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Combined Iron and Folic Acid Supplementation with or without Zinc Reduces Time to Walking Unassisted among Zanzibari Infants 5- to 11-mo old[1,2]

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Abstract

Iron and zinc deficiencies have been associated with delayed motor development in nutritionally at-risk children, albeit inconsistently. In this community-based, randomized double-blind trial, iron+folic acid (FeFA) (12.5 mg Fe + 50 μg folic acid), zinc (Zn) (10 mg), and iron+folic acid+zinc (FeFA+Zn) supplements or a placebo were given daily for 1 y to nutritionally at-risk children in Pemba, Zanzibar. The effects of these treatments on attaining unassisted walking were evaluated using survival analysis for 354 children aged 5–11 mo at the start of supplementation. Treatment effects on changes in hemoglobin (Hb) and zinc protoporphyrin (ZPP) and height-for-age (HAZ) and weight-for-age (WAZ) Z scores were evaluated using linear regression. Attained motor milestone was recorded every 2 wk for 1 y. Hb, ZPP, HAZ, and WAZ were measured at baseline and after 6 mo of treatment. FeFA with or without Zn reduced the time it took for children to walk assisted. Children who received any iron walked unassisted sooner than those who received no iron [median difference ~15 d, $P = 0.035$, risk ratio (RR) = 1.28, 95% CI = 1.02, 1.61] and this effect was stronger in those who had iron deficiency anemia (IDA) at baseline (median difference was ~30 d; $P = 0.002$; RR = 1.68; 95% CI = 1.21, 2.32). FeFA alone and Zn alone improved Hb and ZPP compared with placebo. There were no significant treatment effects on changes in HAZ or WAZ. The effects of treatment on time to walking may have been mediated by improvements in iron status or hemoglobin, but were not mediated through improvements in growth. J. Nutr. 136: 2427–2434, 2006.

Introduction

Walking unassisted is a key milestone in child development. Motor development and motor activity are highly correlated among Indonesian children aged 12 and 14 mo, although the correlation decreases at 18 mo and is close to zero at 24 mo (1). Motor activity contributes to exploration, which, in turn, contributes to the development of visual perception and emotional regulation (2). Independent walking also influences children's interactions with their caregivers (3), which may affect their further development.

It is known that body size, proportion, and muscle and bone strength affect a child's acquisition of locomotion skills (4). Unassisted walking also depends on the acquisition of earlier skills, such as crawling and standing upright. In addition, practice plays an important role in the maintenance and progression of motor development (5). In the case of general malnutrition and iron or zinc deficiency, a child might not have the "energy" or ability to develop new skills or maintain skills already acquired.

Iron deficiency (ID)[8] and iron deficiency anemia (IDA) have been associated with lower scores on global tests of motor development (6–8). Zanzibari children who were neither anemic nor ID were 66% more likely to be walking unassisted than those who were anemic with or without ID (9). In addition, Nepali children who were anemic [hemoglobin (Hb) <105 g/L] were less likely to be walking than those who were not anemic (10). Iron supplementation reduces the prevalence of IDA (11) and improves iron status indicators in ID children (12,13). In addition, one study indicated that iron supplements given to ID anemic children increased their growth (14), although others reported that iron supplementation of iron-replete children has a

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[2] Supplemental Table 1 and Figure 1 are available with the online posting of this paper at jn.nutrition.org.
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[8] Abbreviations used: FeFA, iron+folic acid treatment group; FeFA+Zn, iron+folic acid+zinc zinc treatment group; HAZ, height-for-age Z-score; Hb, hemoglobin; IDA, iron deficiency anemia; MUAC, mid-upper arm circumference; RR, risk ratio; SES, socioeconomic status; WAZ, weight-for-age Z-score; WHZ, weight-for-height Z-score; Zn, zinc supplement group; ZPP, zinc protoporphyrin.

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negative effect on growth (15,16). A recent meta-analysis reported that iron had no significant effect on height or weight growth, although the size of the effect was larger among children who were anemic (Hb <110 g/L) at baseline (17).

Change in length was positively correlated with change in motor development in undernourished populations (18,19). In addition, length was positively related to motor development scores in 12- and 18-mo–old Indonesian children (20). In Zanzibari and Nepali children, stunting was associated with a delay in being able to walk unassisted (9,10). In Guatemala, growth in length and weight during the first year of life predicted the age of attaining unassisted walking (21).

Zinc supplementation improved the growth of zinc-deficient children aged ≥6 mo with height-for-age Z-score (HAZ) <−1.53 (22). It also decreased the incidence of diarrhea, pneumonia (23), and clinical episodes of malaria in some studies (24,25) but not others (26).

Zinc supplementation in infants has been reported to increase motor activity. However, zinc supplementation trials that included global measures of motor development failed to demonstrate the benefit of zinc in terms of motor development, with the exception of one study of low birth weight infants (27). A study of low socioeconomic status (SES) Indian children supplemented with zinc, in addition to other micronutrients, found that children spent more time in high-energy activities and had higher activity scores than those who received the micronutrients without zinc (28). In addition, Guatemalan children who received zinc for 7 mo spent more time sitting up than lying down, and more time playing, than children who received a placebo (29).

The aim of the present study was to evaluate data on 2 key questions: *1*) does daily supplementation with iron+folic acid (FeFA), zinc (Zn) or iron+folic acid+zinc (FeFA+Zn) reduce the time it takes for nutritionally at-risk children to learn to walk unassisted; and *2*) is the response affected by the age at which supplementation is initiated or the child's initial anemia and iron status? Treatment effects, in relation to changes in Hb, zinc protoporphyrin (ZPP), HAZ, and weight-for-age Z-score (WAZ), after 6 mo of supplementation, were also evaluated.

Material and Methods

Description of the study area. Pemba is the smaller of the 2 islands of Zanzibar and is located off of the coast of mainland Tanzania. Malaria is holoendemic and other parasitic infections (including schistosomiasis and hookworm) are common (30). Malaria is transmitted year around; however, the intensity of infection follows a seasonal pattern, with the highest intensities occurring during the period that follows the long rains (Aug–Nov) (31). Children often receive their first exposure to these parasites at a young age. The primary foods consumed by young children in Pemba are breast milk, maize porridge, rice, and fried dough *(andazi)*, tea, and tastes of fruits and family dishes.

Study design. The present research was part of the Child Development Substudy of a larger trial in Pemba designed to determine the effects of FeFA, Zn, or FeFA+Zn on morbidity and mortality among children aged 1–35 mo (32). The substudy was designed to determine the effects of these treatments on child motor development and activity and language and social development. In the main trial, children were randomized to receive iron (12.5 mg) + folic acid (50 μg) (FeFA), zinc (10 mg) (Zn), FeFA+Zn, or a placebo daily for 1 y. Children <12 mo received one-half of the dose. If children were found to be severely anemic (Hb <70 g/L) they were treated with 25 mg Fe, 100 μg FA, and 1 μg vitamin B-12 daily for 3 mo and were further randomized to receive mebendazole or not, and continued with regular supplements. The proportion of children who received this additional treatment at any time (baseline, after 3, 6, or 9 mo of supplementation) was not significantly different across the 4 supplement groups (FeFa = 28.1%, Zn = 38.9%, FeFA+Zn = 26.7%, placebo = 23.3%). This study received ethical approval from the institutional review boards of Johns Hopkins University, University of California at Davis, Cornell University, and the Zanzibar Ministry of Health.

Subjects and enrollment. All children in Pemba aged 1–35 mo were invited to participate in the main trial. Pemba is divided into 4 districts and was further divided into neighborhoods for this study. Wete District was chosen as the site for this substudy, and neighborhoods within Wete were selected based on geographic convenience. Urban and rural areas are represented in this sample. All children aged 5–18 mo at enrollment, living in the included neighborhoods, and whose parents agreed to their participation in the main trial, were asked to participate in the Child Development Substudy. Oral consent was obtained from the primary caregiver at the time of enrollment. Of 932 children initially enrolled in the Child Development Substudy, 56 did not participate in the baseline health clinic or home visit assessments, including observations and parental interviews, and were therefore excluded from the substudy.

Exclusion criteria: time to walking sample. The primary outcome of these analyses was the time it took for children to walk unassisted. The cross-sectional age of children who were reported to be walking unassisted at the start of the study was 15.9 ± 2.2 mo (mean ± SD). To avoid including children who may have started walking late for reasons unrelated to nutritional status, those who were ≥12 mo of age at baseline were excluded from the analyses (n = 471). In addition, children were excluded if they were missing baseline data on the motor milestone (n = 22), Hb and/or HAZ (n = 5) status, were walking unassisted at either the first or second milestone visit (n = 13), did not have at least 4 complete milestone visits (n = 3), or were missing >2 visits between the milestone prior to walking unassisted and the visit when walking unassisted was recorded (n = 8) (**Figure 1**).

Exclusion criteria: biochemical and anthropometric outcomes. To evaluate the treatment effects of FeFA, Zn, and FeFA+Zn on Hb, ZPP, and growth, children needed to complete both the baseline and 6-mo assessments. Children who were missing 6-mo assessments of Hb (n = 17), both Hb and HAZ (n = 1), or all 6-mo clinic data (n = 123) were excluded from all analyses. Those who were missing the baseline assessment of ZPP (n = 14), 6-mo assessment (n = 27), or both (n = 8) were excluded only from the analyses involving that particular measure. More children were missing ZPP because of occasional problems with the hematofluorometer. Analysis using ANOVA for continuous variables and chi-square for dichotomous variables did not significantly differ in baseline values of Hb, ZPP, HAZ, or WAZ, age, or SES between those attending and not attending the 6-mo clinic (Figure 1).

Measures and procedures. Once the child was enrolled in the substudy a home visit was scheduled. At the home visit, baseline motor milestone was assessed using a picture chart (**Supplemental Fig. 1**), an observation was conducted and other interviews about the child's appetite, sleep, motor, and language development were administered. After completing all of these measures, the child was scheduled to visit the health clinic for a blood draw and anthropometric measures; 74% of the health clinic visits occurred within 1 wk of the observation and 95% within 2 wk. Children received their first packet of supplements at the health clinic, and home monitors delivered supplements weekly for the remainder of the trial.

For this substudy, children were visited in their homes by a trained observer every 2 wk for 1 y or until they attained the highest milestone on the chart (standing on one foot), whichever came first. Observations and the aforementioned interviews were conducted every 3 mo, at the child's home, for a total of 5 assessments on these measures; health clinic visits occurred at baseline, 6 mo, and 12 mo.

Attained motor milestone. The highest motor milestone that a child achieved was recorded following the observation. Motor development was assessed from a picture chart containing 14 gross motor milestones that were used previously (33) and was based on the work of McGraw (34). The chart was shown to the primary caregiver at the baseline visit,

and each of the milestones was described one at a time. Following each description the caregiver was asked if she had seen her child perform that milestone [see Kariger et al. (9) for description of milestones]. Once the caregiver answered no to a milestone, the previous milestone was recorded as the highest one attained and reported by the caregiver. The child was then asked to demonstrate the activity. If the child demonstrated the activity, the observer encouraged the child to do the next highest milestone and continued until the child could no longer demonstrate a milestone. If the child could not do the milestone reported he or she was asked to try the previous one, continuing until the child successfully demonstrated a milestone. The highest attained milestone was then recorded as the highest one demonstrated. When the parental report differed from the highest demonstrated milestone, the demonstrated milestone was used in the analyses.

Clinical assessment. Venous blood (3–5 mL) was collected at the clinic visits and divided into 3 parts. Whole blood was used immediately to measure Hb concentration (Hemocue, AB) and to prepare thick and thin blood films. The blood films were transported to the laboratory where they were fixed, stained with Gimesa, and examined for malaria parasites. The second aliquot was used to measure ZPP (hematofluorometer, Aviv Biochemical) and total leukocyte count. The third aliquot of blood was transferred into a heparinized zinc-free polypropylene tube and transported in a cooler to the lab where it was centrifuged (1500 × *g* for 10 min) and stored in a –70°C freezer for later analyses. These measures were taken at baseline and after 6- and 12-mo of treatment.

Length, weight, and mid-upper–arm circumference (MUAC) were measured before the blood draw using standard methods by a trained staff. Recumbent length was measured to the nearest 0.1 cm using a wooden length board (Shorr Productions). MUAC was measured to the nearest 0.1 cm with a flexible insertion tape, and weight was obtained to the nearest 0.1 kg using a digital scale (Seca Scales). Length and MUAC measurements were performed in triplicate and weight in duplicate; mean values were used for analysis. Epinut (Epi Info 2002, CDC) was used to calculate HAZ, WAZ, and weight-for-height Z-score (WHZ) using the WHO 1978 reference charts.

Socioeconomic status. The socioeconomic status (SES) variable used in this analysis was a composite score based on parental responses to a questionnaire. The questionnaire included questions about personal and family resources (annual cash income, parental education, and father's employment) and the quality of the home (wall type, floor type, and source of water). For a more detailed discussion of the construction of this variable see Kariger et al. (9).

Statistical analysis. The primary objective of these analyses was to examine whether treatment with daily FeFA, Zn, or FeFA+Zn reduced the time to walking among children aged 5–11 mo from the start of supplementation. Two secondary objectives were to examine if the effect of supplementation was different according to baseline age or baseline IDA status [defined as Hb <100 g/L and ZPP ≥90 μmol/mol heme (35)]. Since motor milestones were only assessed every 2 wk, time to walking was defined as the number of days the child had been taking supplements when unassisted walking was first observed, minus one-half of the days since the previous visit. Cox's proportional hazard models were used to test if supplementation reduced the time it took for children to walk unassisted.

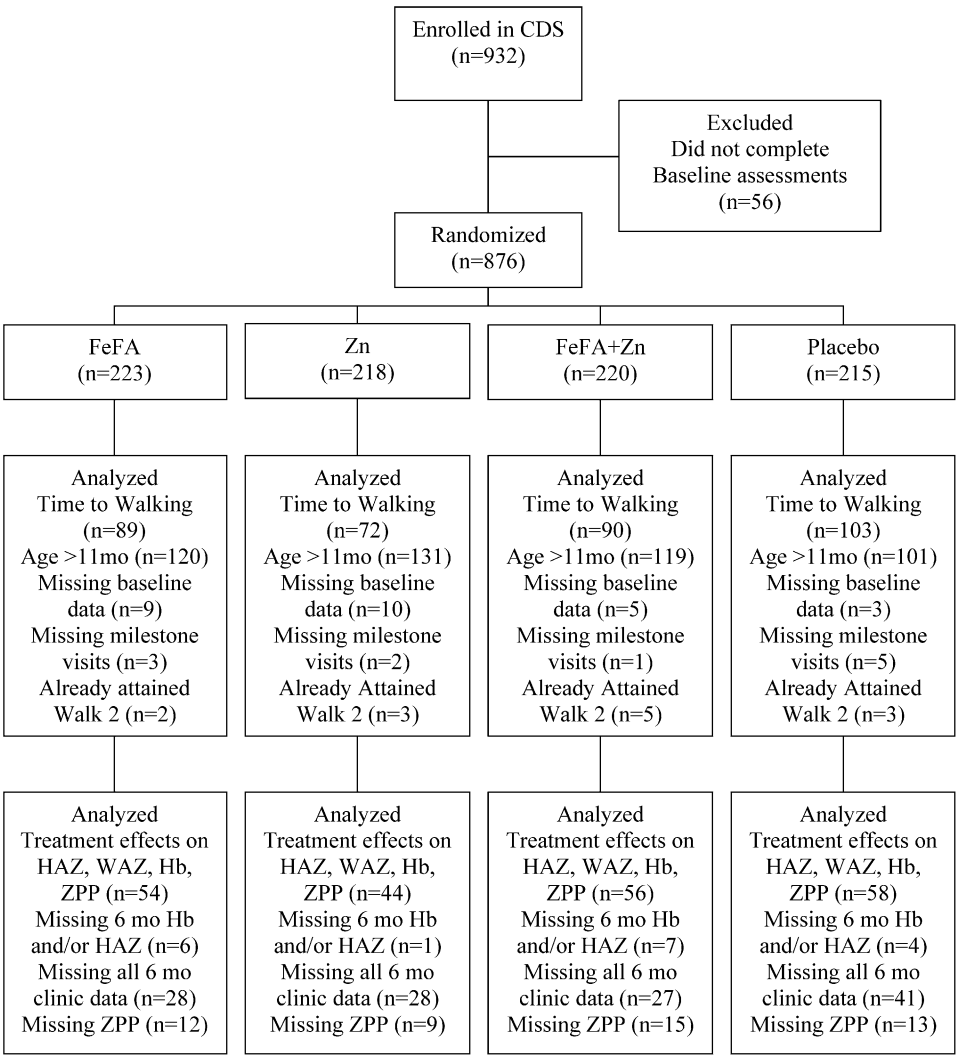

Figure 1 Child development substudy (CDS): number of children in each supplement group and included in time to walking and change in Hb, ZPP, HAZ, and WAZ analyses.

The primary analysis examined the main effects of FeFA [with or without Zn (any iron)] and Zn [with or without FeFA (any zinc)] and included the interaction any iron × any zinc. To examine if baseline age influenced response to treatment, a 3-way interaction term was included (any iron × any zinc × age squared). To test if baseline iron deficiency anemia status influenced the response to iron, an interaction term (baseline IDA status × any iron) was included in a separate model. When interactions were significant, results were presented as main effects or groups represented by the interaction (i.e., IDA baseline, received FeFA, no IDA baseline, no FeFA, etc.). For the any iron × any zinc × age squared interaction, results were stratified by age group (5–7, 8–9, and 10–11 mo). Risk ratios (RR) and 95% CI were calculated based on Cox proportional hazard models. The RR compared the rate of walking among the treatment groups. A risk ratio >1 indicated that the time to walking is shorter. The difference in days to walking reported were based on the number of days at which 50% of treatment group children were walking. All models included gender, SES, age, Hb, HAZ, and attained milestone at baseline as covariates. Variables were considered significant, $P \leq 0.05$ and $P < 0.10$ for interaction terms.

To examine whether the treatment effects on time to walking could be explained by changes in Hb, ZPP, HAZ, or WAZ, linear regression was used to assess the effects of FeFA, Zn, and FeFA+Zn on changes in these measures from baseline to 6 mo. Logistic regression was used to examine differences in the prevalence of Hb <100 g/L, ZPP ≥90 μmol/mol heme, and malaria (positive blood film). Baseline status, gender, age, and SES were included as covariates in all models. Baseline malaria status was included initially but did not contribute significantly to any of the models.

Baseline characteristics were compared among groups using ANOVA for continuous variables and chi-square for categorical variables. Baseline motor milestones were evaluated separately, comparing the proportion of children at each milestone by treatment group. Values in the text are means ± SD unless noted otherwise.

'SPSS, version 13.0, was used for all analyses.

Results

Baseline characteristics. The mean age of children was 8.8 ± 1.9 mo, and 50.8% were male (**Table 1**). The Hb concentration for the sample was 92 ± 16 g/L, and HAZ was –1.4 ± 1.1. The prevalence of anemia (Hb <100g/L) was 63%, ID (ZPP ≥90 μmol/mol heme) 78%, and IDA (Hb <100g/L and ZPP ≥90 μmol/mol heme) 58%. Thirty-one percent of the children had a positive malaria smear at baseline. Treatment groups did not differ at baseline, with the exception of baseline Hb. Children in the FeFA group had significantly lower Hb concentrations than those in the placebo group.

Effects of treatment on time to walking. A main-effects model was used to examine the main effect of iron with or without zinc (any iron group) and Zn with or without FeFA (any zinc group) on the attainment of the ability to walk unassisted. The interaction term any iron × any zinc was also included, and gender, SES, age, Hb, HAZ, and attained milestone were entered as covariates. In this model, the interaction term was significant ($P = 0.035$, RR = 1.65, 95% CI = 1.04, 2.63). Children who received any iron walked unassisted sooner than those who received no iron (**Table 2**, **Fig. 2***A*).

To examine the influence of baseline age on the treatment effects seen in the first model, a 3-way interaction term was included (any iron × any zinc × age squared) and this was significant ($P = 0.054$, RR = 0.99, 95% CI = 0.97, 1.00). Children were then stratified by age (5–7, 8–9, and 10–11 mo at the start of supplementation) and main-effect results were presented (Supplemental Table 1). Supplementation with FeFA with or without Zn from the age of 5–7 mo caused children to walk ~1 mo earlier than those who received no iron (median ~220 d for the any iron group and ~252 d for the no-iron group, $P = 0.020$, RR = 1.64, 95% CI 1.08, 2.49). There were no significant treatment effects among children who were aged 8–9 mo at the start of supplementation. The oldest children at baseline (10–11 mo) also benefited from FeFA with or without Zn (median ~110 d in the any iron group and ~122 d in the no-iron group, $P = 0.055$, RR = 1.50, 95% CI 0.99, 2.27).

To examine whether these effects were stronger among the children who were iron deficient anemic at baseline, the first model was used including an interaction term, any iron × baseline IDA status (**Table 3**, **Fig. 2***B*). The interaction term was significant ($P = 0.030$, RR = 1.76, 95% CI = 1.07, 2.89). Children

TABLE 1 Baseline characteristics of children by supplement group[1]

	FeFA, $n = 89$	Zn, $n = 72$	Zn+FeFA, $n = 90$	Placebo, $n = 103$
Age, *mo*	8.7 ± 1.8	9.0 ± 1.8	8.6 ± 1.9	9.0 ± 1.8
Male, *%*	48.3	47.2	56.7	50.5
Socioeconomic status	12.7 ± 4.3	13.1 ± 4.8	13.1 ± 4.6	13.0 ± 5.5
Motor milestone[2]	Crawl	Stand 1	Creep 2	Crawl
Hemoglobin, *g/L*	89.4 ± 15.3*	91.0 ± 15.6	92.2 ± 14.6	95.6 ± 17.2
Anemic,[3]%	68.5	62.5	63.3	59.2
Zinc protoporphyrin,[4] *μmol/mol heme*	195.4 ± 126.7	197.5 ± 171.5	179.3 ± 127.5	191.8 ± 119.5
Iron deficient,[5]%	82.3	71.4	75.0	82.3
Iron deficient anemic,[4,6]%	63.3	54.0	57.9	55.2
Malaria,[7]%	36.0	27.8	30.3	29.1
Height-for-age Z score	−1.5 ± 1.0	−1.3 ± 1.0	−1.4 ± 1.2	−1.6 ± 1.0
Weight-for-age Z score	−1.2 ± 1.3	−1.1 ± 1.1	−1.1 ± 1.2	−1.4 ± 1.1
Weight-for-height Z score	−0.03 ± 1.1	−0.1 ± 1.1	−0.09 ± 1.1	−0.2 ± 1.1

[1] Values are expressed as means ± SD, or percentages unless otherwise noted. * Different from placebo, $P \leq 0.05$.
[2] Median achievement level.
[3] Anemia defined by Hb <100 g/L.
[4] FeFA, $n = 79$; Zn, $n = 62$; Zn+FeFA, $n = 75$; Placebo, $n = 96$.
[5] Iron deficiency defined by ZPP ≥90 μmol/mol heme,
[6] Iron deficiency anemia defined by Hb <100 g/L and ZPP ≥90 μmol/mol heme,
[7] Positive blood smear.

TABLE 2 Risk ratios (RR) obtained from Cox proportional hazard models based on the main effects of any iron and any zinc on time to walking for all children[1]

	RR[2]	95% CI for RR
Any iron (FeFA and FeFA+Zn)	1.28*	(1.02, 1.61)
Any zinc (Zn and FeFA+Zn)	0.91	(0.73, 1.15)
Hemoglobin	1.07	(0.99, 1.16)
Height-for-age Z score	1.20*	(1.07, 1.34)
Motor milestone	1.38*	(1.29, 1.48)
Age	1.28*	(1.18, 1.38)
Gender	0.97	(0.77, 1.23)
Socioeconomic status	1.06*	(1.03, 1.09)

[1] Any iron × any zinc interaction was significant, $n = 354$; $P = 0.035$, RR = 1.65, 95% CI = 1.04, 2.63.
[2] RR >1 indicates shorter time to walking, *$P \leq 0.05$.

TABLE 3 Risk ratios obtained from Cox proportional hazard models based on the interaction of any iron × iron-deficiency anemia status in children at baseline[1]

	RR[2]	95% CI for RR
Noniron-deficient anemic, no iron[3]	1.54	(0.97, 2.43)
Noniron-deficient anemic, any iron[3]	1.48	(0.95, 2.33)
Iron-deficient anemic, any iron[3]	1.68 *	(1.21, 2.32)
Zinc	1.02	(0.80, 1.31)
Hemoglobin	1.02	(0.90, 1.16)
Height-for-age Z score	1.21 *	(1.07, 1.37)
Motor milestone	1.40 *	(1.30, 1.50)
Age	1.32 *	(1.20, 1.44)
Gender	1.02	(0.79, 1.32)
Socioeconomic status	1.05 *	(1.02, 1.08)

[1] Groups based on significant interaction (any iron × iron deficiency anemia status at baseline), $P = 0.030$, RR = 1.76, 95% CI = 1.07, 2.89.
[2] RR >1 indicates shorter time to walking.
[3] Reference group = iron-deficient anemic, no iron, *$P \leq 0.05$.

who were IDA at baseline and received any iron walked ~30 d earlier than those who received no iron.

Effects of treatment on anemia, iron deficiency and anthropometry. The observed treatment effects might be mediated in part by improvements in iron status and/or linear growth. To explore this, a linear regression model of the treatment effects on the change in Hb, ZPP, HAZ, and WAZ from baseline to 6 mo ($n = 150$) was used. Gender, SES, and baseline values of age and the respective biochemical or anthropometric values were included as covariates. In the Hb and ZPP models the iron × zinc term was significant, but the main effects were not. There was a significantly greater reduction in the prevalence of anemia (Hb <100 g/L) in the children who received FeFA+Zn compared with the placebo. Changes in the prevalence of iron deficiency among treatments did not differ (ZPP ≥90 μmol/mol heme) (**Table 4**). There were no significant treatment effects on changes in HAZ or WAZ. Prevalence of malaria (positive blood film) was examined among groups at baseline and at 6-mo by logistic regression and did not differ as a result of treatment (data not shown).

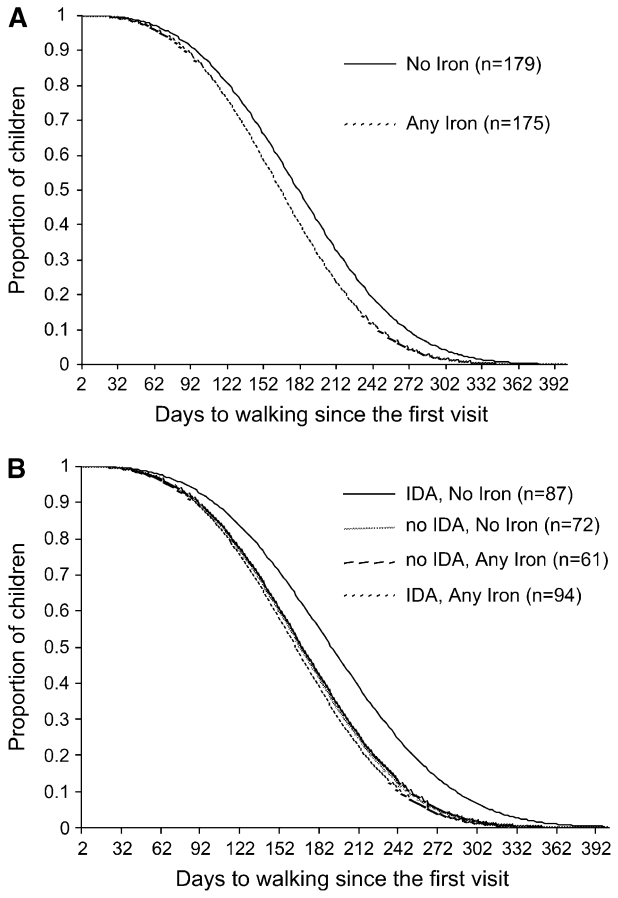

Figure 2 Effect of iron with or without zinc (any iron) compared with zinc and placebo (no iron) on time to walking in all children (*A*) and in children with and without iron deficiency anemia at baseline (*B*). Iron deficiency anemia = Hb <100 g/L and ZPP ≥90 μmol/mol heme. Cox proportional hazard models controlling for gender, SES, age, Hb, HAZ, and attained milestone at baseline.

Discussion

Iron+folic acid supplementation alone or in combination with zinc reduced the time to walking and this effect was stronger among the youngest children and in children with iron deficiency anemia at baseline. The data indicated that treatment effects on

TABLE 4 Effects of supplements on anemia, iron deficiency, and anthropometry in 4 groups of children over a period of 6 mo[1]

	FeFA, $n = 54$	Zinc, $n = 44$	Zinc+FeFA, $n = 56$	Placebo, $n = 58$
Hemoglobin, *g/L*	4.2 ± 11.9*	3.4 ± 11.5*	1.3 ± 10.9	−7.5 ± 11.4
Anemia,[2,3]%	66.7	65.9	57.1*	75.9
Zinc protoporphyrin,[4] *μmol/mol heme*	−28.3 ± 81.7*	−49.8 ± 113.0*	−21.5 ± 101.1	13.7 ± 67.0
Iron deficient,[2,4,5]%	76.7	74.3	76.2	81.4
Height-for-age Z score	−0.32 ± 0.25	−0.43 ± 0.26	−0.34 ± 0.25	−0.35 ± 0.26
Weight-for-age Z score	−0.17 ± 0.54	−0.22 ± 0.46	−0.28 ± 0.49	−0.15 ± 0.44

[1] Values are means ± SD or percentages; *Different from placebo, $P \leq 0.05$. Changes are from baseline to 6 mo unless otherwise noted.
[2] Prevalence at 6 mo.
[3] Anemia defined by Hb <100 g/L.
[4] FeFA, $n = 43$; Zinc, $n = 35$; FeFA+Zn, $n = 42$; Placebo, $n = 43$.
[5] Iron deficiency defined by ZPP ≥90 μmol/mol heme.

the time to walking might have been mediated by improvements in Hb or iron status but were not mediated through improvements in linear growth (Table 4).

The treatment effects reported here are attributed to the iron portion of the supplement rather than the folic acid because of the prevalence of iron deficiency anemia (57.6%) and the improvements seen in hemoglobin and ZPP concentrations with FeFA supplementation. Furthermore, in another study done in this community, the mean serum folate concentration of children 12–18 mo old ($n = 42$) was 25.22 ± 9.00 nmol/L (Kimberly O'Brien, Cornell University, personal communication). Values <6.8 nmol/L indicate low serum folate (36).

FeFA alone and Zn alone improved Hb and ZPP concentrations. It was expected that iron would improve iron status but not hemoglobin, based on previous work in this population (13). Improved Hb and ZPP in the Zn group may be the result of improvements in appetite, immune function, or in the inflammatory response to parasites. The children who received FeFA+Zn showed some improvement in Hb and ZPP compared with the placebo groups, but the differences were not significant. Iron and zinc, when given together, produced limited improvements in Hb compared with iron given alone in some studies (37,38) but not in others (39). For a review see Fischer et al. (40).

Iron supplementation improves motor development as measured by global tests of development (41). However, this improvement has not been consistently demonstrated (42). A randomized placebo controlled trial of Zanzibari preschoolers revealed that children who had Hb <90 g/L and received an iron supplement had higher scores on a motor-development scale than those who received a placebo (13). Furthermore, reversals of delays in motor development related to IDA have been demonstrated in response to iron supplementation (43,44).

Iron+folic acid supplementation, with or without zinc, reduced the time it took children to learn to walk unassisted in the study population. One mechanism through which iron supplementation may benefit motor development is through improving the anemia or iron deficiency status of the individual. This, in turn, may alleviate some of the negative symptoms associated with iron deficiency, including lethargy and withdrawal. Improved iron status increases the oxygen-carrying capacity of the blood, which may lead to more motor activity.

Another possibility is that improvements in iron status may improve myelination, which increases the speed at which information is processed. Auditory brainstem responses were compared between nonanemic and iron-deficient anemic 6-mo–old Chilean children. The IDA children had longer central conduction times than their nonanemic controls at 6 mo and the differences persisted at 12 mo and 18 mo (12). In addition, pups of Sprague Dawley rat dams fed an iron deficient diet from early gestation until 20 d after birth, and then fed an iron sufficient diet during weaning until they were 6-mo old, did not have normal myelin composition compared with controls (45).

In addition to these improvements at the level of the child, iron supplementation may confer additional benefits by changing the caregiver's perception and interactions with a child. Parental encouragement and motivation play a role in the acquisition of new motor skills. The caregiver may encourage a child, seen as healthy, to practice already acquired skills and to learn new skills, which increases the child's motivation. Costa Rican iron-deficient anemic infants, aged 12–23 mo, were encouraged less by testers to perform tasks during motor testing using the Bayley Scales than children who were not iron-deficient anemic. In addition, the parents of the iron-deficient anemic children were less likely to be rated as highly affectionate during both the motor and mental parts of the Bayley Scales compared with parents of children who were not iron-deficient anemic (46).

It is unclear why iron treatment given to children 8–9 mo of age did not hasten walking, as seen in the younger (5–7 mo) and older (10–11 mo) children. Children in this population are exposed to many different risk factors to optimal development, including a low quality diet, poor access to health care, infections that include malaria and helminthes, and symptoms related to iron and zinc deficiency, including poor appetite and impaired immune function.

One explanation as to why treatment effects were not significant in the 8–9 mo group may be seasonality. In Pemba, malaria is transmitted year around, although the intensity of infection follows a seasonal pattern with the highest intensities occurring during the period following the long rains (July–Aug) (31). This study began in March 2001 and children were enrolled until the end of May 2001. Due to the age at enrollment (8–9 mo) and the average age of walking (15 mo), children in this age group would have been expected to begin walking unassisted directly after the period of highest intensity of malaria infection. In other related analyses with this sample of children, those with malaria infection (slide positive), and who were yet unable to walk, had significantly lower total motor activity scores and spent less time in locomotion than those who did not have a malaria infection (D. K. Olney, unpublished data). Given that practice and motivation play a role in the acquisition of motor skills, and that malaria infection was the strongest predictor of hemoglobin concentration in children aged <30 mo in this population (30), it is possible that treatment with iron+folic acid alone, or zinc alone or in combination was not enough to overcome the negative impact of malaria infection during this time period.

In this study, zinc given alone did not reduce the time it took for children to walk alone. Zinc supplementation trials that have included global measures of motor development have failed to show a benefit (29,47–49), with the exception of one study of very low birth weight infants (27). However, other benefits of zinc supplementation related to child development have been reported, including higher motor activity scores (28) and spending more time in play and sitting up rather than lying down (29), but the significance of these findings to overall child development are not clear.

Walking alone is a key milestone attainment and represents a normative shift in child development. The importance of this milestone in relation to other areas of child development has been explored. Early walkers demonstrate changes in their interactions with their mothers, representing autonomy and assertiveness (3). In addition, early walkers are more sociable and more likely to have affectionate relationships with their mothers (50). Unassisted walking also increases the child's opportunities for exploration. In other related analyses, total motor activity scores were strongly correlated with motor development as measured by the motor milestone scale.

In this population where anemia, iron deficiency, and iron deficiency anemia are highly prevalent, supplementation with iron+folic acid with or without zinc reduced the time it took for children, aged 5–11 mo at the start of supplementation, to learn to walk unassisted. Furthermore, the benefits of iron supplementation were stronger among children who were iron deficient and anemic at the beginning of the study. The evidence presented in this paper, in addition to what others have reported, leads to the conclusion that iron+folic acid supplementation of iron-deficient anemic children improves motor development (43,44).

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